Residual Stress in Bone Structure and Tissue of Rabbit's Tibiofibula

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ABSTRACT

This paper presents an X-ray diffraction method of measuring the residual stress/strain in bone tissue of rabbit's tibia. To derive the residual stress, bone powder of the diameter less than 40 micrometer was used as a control specimen at non-stressed state. From the X-ray measurements, it was clear that the distribution of residual stress existed in the bone tissue. The tensile residual stress at bone axial direction occurred in the proximal-medial region of rabbit's tibia. The compressive stress occurred in the other regions. In addition, the mechanism to generate the residual stress was investigated by sequential cutting of the tibiofibula system from bone structure scale to bone tissue scale. The remodeling is a phenomenon that the bone structure adapts functionally to mechanical environment. The residual stress will become a mechanical trigger to induce the remodeling.

KEYWORDS

Biomechanics, Residual Stress, X-ray Diffraction, Bone Structure, Bone Tissue, Rabbit's Tibia, Bone Remodeling

1. INTRODUCTION

It is well known that bone has a capability to adapt to additional mechanical environment and to reconstruct functionally its structure and geometry [1][2][3]. This phenomenon is called "adaptive bone remodeling" or "Wolff's Law". One of the most important mechanical factors of remodeling is the stress [4]. To generate the bone remodeling, some stress should be present in bone tissue to stimulate the osteocyte over a relatively long period. Thus stress will be a type of residual stress that satisfies a new equilibrium of force in bone tissue [5]. To verify this assumption, it is necessary to measure the residual stress in intact bone tissue.

Fung [6] suggested the importance of the existence of residual stress in living tissue. Residual stress in soft tissue was measured using the arterial wall as a specimen [6][7]. In hard tissue, Tanaka and Adachi [8][9] reported that residual strain in leporine tibia bone was examined with a strain gauge application, by cutting the fibula from tibiofibula of a statically indeterminate structure.

The X-ray diffraction method is effective to measure nondestructively the stress/strain in crystal materials. Authors have developed a method to measure residual stress in artificial hydroxyapatite ceramics. The application of which has been reported on such biomaterials as sintered hydroxyapatite [10] and hydroxyapatite coating titanium implant [11]. Since bone tissue also includes hydroxyapatite crystals, authors have also proposed a polychromatic X-ray method to measure the anisotropic residual stress in bovine femoral bone, and represented the existence of residual stress in intact bone [12].

Experimental technique using the characteristic X-ray is more simple and easier than the polychromatic X-ray method. This paper presents a measurement method for the residual stress/strain in rabbit's tibia using the characteristic X-ray. Therefore, it was proved that the residual stress/strain distributed in the tibia. In addition, the mechanism to induce residual stress/strain was investigated on macroscopic structure of

tibiofibula and microscopic structure of bone tissue.

2. X-RAY DIFFRACTION METHOD FOR BONE TISSUE

The main mineral component of bone tissue is hydroxyapatite (HAp: $Ca_{10}(PO_4)_6(OH)_2$). Because HAp is a crystal structure, X-ray diffraction method is available to measure the strain of HAp crystals [12]. Bragg's law as the fundamental equation of X-ray diffraction is expressed as equation (1).

$$\lambda = 2d\sin\theta \tag{1}$$

Using the characteristic X-ray with a unique wavelength λ, equation (1) relates the diffracted angle θ to the interplanar spacing d at a specific lattice plane (hkl) as shown in Figure 1. Because there are so many the same lattice planes in a material, the value of θ detected is related to the mean value of d. Deforming bone tissue, interplanar spacing d at the lattice plane in it changes also. The mean strain ε is defined as the ratio of d at strained state and d_0 at non-strained state. From equation (1), the strain is formulated as equation (2) by θ_0 and θ measured at non-strained and strained specimen, respectively.

$$\varepsilon = \frac{d - d_0}{d_0} = \frac{\sin\theta_0 - \sin\theta}{\sin\theta} \tag{2}$$

This method applies to compact bone with relatively high crystallinity of HAp. Compact bone is an orthotropic material reinforced to a bone axis. The coordinate system of bone is defined as shown in Figure 2. The x, y and z-axis correspond to the bone axial, circumferential and radial direction, respectively. The origin of coordinate puts on a point at a bone surface. The strain ε_i at arbitrary i-direction measured by X-ray diffraction is formulated by three normal strain components as equation (3).

$$\varepsilon_i = l_i^2\varepsilon_x + m_i^2\varepsilon_y + n_i^2\varepsilon_z \tag{3}$$

Where l_i, m_i and n_i are direction cosines. Three strains ε_x, ε_y and ε_z are calculated from each strain (i = 1, 2, 3) measured independently at arbitrary three directions. In this

experiment, these directions were selected as follows;

$$\varepsilon_1 = \varepsilon_x \sin^2\psi + \varepsilon_z \cos^2\psi, \quad (\sin\psi, 0, \cos\psi) \tag{4}$$

$$\varepsilon_2 = \varepsilon_y \sin^2\psi + \varepsilon_z \cos^2\psi, \quad (0, \sin\psi, \cos\psi) \tag{5}$$

$$\varepsilon_3 = \varepsilon_z, \quad (0, 0, 1) \tag{6}$$

On the bone axial direction as shown in Figure 2(a), the symbol ψ indicates the angle between a plane including X-ray path at y = 0 and z- axis. The bone axial strain ε_x is derived from a couple of lattice strain ε_1 and ε_3 (= ε_z) measured at directions of ($\sin\psi$, 0, $\cos\psi$) and (0, 0, 1). On the circumferential direction as Figure 2(b), the angle ψ becomes the value between a plane including X-ray path at x = 0 and z-axis. The circumferential strain ε_y is derived from a couple of lattice strain ε_2 and ε_3 measured in each directions of (0, $\sin\psi$, $\cos\psi$) and (0, 0, 1). Where the strain ε_3 (= ε_z) is measured at the perpendicular plane (0, 0, 1) on the bone surface. From equations (4)-(6), strain components can be represented as equations (7) and (8). In this experiment, the value of angle ψ was selected as 30 deg.

$$\varepsilon_x = \frac{\varepsilon_1 - \varepsilon_3 \cos^2\psi}{\sin^2\psi} = \frac{4\varepsilon_1 - \varepsilon_3}{3} \tag{7}$$

$$\varepsilon_y = \frac{\varepsilon_2 - \varepsilon_3 \cos^2\psi}{\sin^2\psi} = \frac{4\varepsilon_2 - \varepsilon_3}{3} \tag{8}$$

Since X-ray is diffracted from a very thin layer below 50 micrometer at bone surface [12], the stress at bone surface is assumed to be in a plane stress state. Bone axial stress σ_x and circumferential stress σ_y are then derived using equations (7) and (8) as equations (9) and (10), respectively.

$$\sigma_x = \frac{\varepsilon_1 - (N_2 \sin^2\psi + 1)\varepsilon_3}{N_1 \sin^2\psi} = \frac{4\varepsilon_1 - (3N_2 + 4)\varepsilon_3}{3N_1} \tag{9}$$

$$\sigma_y = \frac{\varepsilon_2 - \varepsilon_3}{N_3 \sin^2\psi} = \frac{4(\varepsilon_2 - \varepsilon_3)\varepsilon_1}{3N_3} \tag{10}$$

Where N_1, N_2 and N_3 are material constants represented by Young's modulus E and

Poisson ratio ν, as equations (11) to (13).

$$N_1 = \frac{1}{E_x}\left(1 + \frac{\nu_{yx}\nu_{xy}}{\nu_{yz}}\right) \tag{11}$$

$$N_2 = \frac{\nu_{yx}}{\nu_{yz}} - 1 \tag{12}$$

$$N_3 = \frac{1}{E_y}\left(1 + \nu_{yz}\right) \tag{13}$$

3. DETERMINATION OF MATERIAL CONSTANTS

In order to calculate the stress σ_x and σ_y of equation (9) and (10), material constants of N_1, N_2 and N_3 in equations (11) to (13) should be determined in advance using the four-point bending device [12]. This device is illustrated in Figure 3, which is designed to be used in the irradiation area of the X-ray diffraction system. For that, the size of specimen needs 28x10x2 mm. Because of a very small diameter of rabbit's tibia, it was difficult that a specimen was made with their long axis aligned to the circumferential direction. Therefore, the stress σ_y is not derived in this work. A strain gauge was bonded to the opposite side of the specimen surface irradiated by X-ray. When the strain ε_x^* is measured by the strain gauge in the bone axial direction, the stress on the surface irradiated by X-ray are obtained simply as.

$$\sigma_x = -E_x\varepsilon_x^* \tag{14}$$

Where E_x is the elastic modulus for bone axial direction. This modulus was obtained from the measurements of macroscopic stress and strain by the other four-points bending test. On the assumption of unique elastic constants in compact bone or rabbit's tibia, a strip specimen was cut from diaphysis of tibia. Therefore, the value of E_x was obtained as 25.92±2.01 GPa (mean±S.D., n=3).

Here the bone axial stress σ_x is only derived. From equations (9) and (14), the

following equation is obtained:

$$\frac{\varepsilon_1/\varepsilon_3-1}{\sin^2\psi}=-\frac{E_x\varepsilon_x^*}{\varepsilon_3}N_1+N_2 \tag{15}$$

$$4(\varepsilon_1-\varepsilon_3)=-3E_x\varepsilon_x^*N_1+3\varepsilon_3N_2 \tag{16}$$

. The material constants N_1 and N_2 can be determined using the least squares method from the values of ε_1 and ε_3 measured from X-ray diffraction method while the specimen is deformed under stepwise loading. Therefore, the values of N_1 and N_2 were obtained as -2.16±0.52 (mean±S.D., n=3) and -5.38±1.71, respectively.

4. SPECIMEN

Fresh shanks were excised from three healthy Japanese white rabbits of 2.5±0.2 kg weight (mean±S.D., n=3). The tibia and fibula in shank are interconnected in a statically indeterminate structure as shown in Figure 4. X-ray was irradiated at each anterior, posterior, lateral and medial point in proximal region of 25 mm from the upper epiphysis, in intermediate region of the diaphysis and in distal region of 20 mm from the lower epiphysis. To examine the effect of release in the indeterminate structure or bone tissue on residual stress, specimens at every condition were made by sequential cutting from intact shank. These specimens are shown in Figure 5. No. C0 is intact shank covered with soft tissue cut hair. C1 is tibiofibula bone removed the soft tissue of muscle and skin from C0. C2 is tibia bone removed fibula from C1. C3 is diaphysis of tibia removed both epiphyses from C2. C4 is three parts (proximal, intermediate and distal) cut from C3. C5 is four parts cut at bone axial direction (anterior, posterior, medial and lateral) from each C4.

To investigate the effect of microscopic structure in bone tissue, bone powder was made from the same compact bone. These particle sizes are over 1000 mm, 500-1000 mm, 90-500 mm, 45-90 mm and below 45mm mean diameter.

5. EXPERIMENTAL METHOD

Figure 6 shows the characteristic X-ray diffraction system (RINT 2200, Rigaku Co., Japan) used in this experiment. A bone specimen was mounted in the center on a goniometer. Table 1 lists condition of X-ray diffraction. Monochromatic X-ray beam is generated at the X-ray generator with Molybdenum tube. The detective counter is mounted on a goniometer, which can be rotated about the axis of the center of specimen. Goniometer and specimen are mechanically coupled such that a rotation of the specimen through θ is accompanied by a 2θ rotation of the counter. This assures that the incident and diffraction angles are maintained equal to one another. As the counter moves at constant angular velocity of 3 deg/min, an on-line computer system automatically records the diffracted beam intensity (monitored by the counter) as a function of 2θ that is termed the diffracted angle.

Because a crystal structure of HAp in compact bone has much lower crystallinity than metal as steel, the 2θ angle to correspond to the peak position (maximum value) of diffracted intensity is not easy to determine from an intensity-angle profile. Therefore, this method was used the combined profile with lattice planes of (211), (112), (300) and (202) of HAp crystal in bone tissue. Figure 7 shows a typical relationship between intensity and diffracted angle. As shown in this figure, the peak position was determined by removing the influence of background intensity and applying the FWTTM method (Full Width of Two Third Maximum Intensity).

On the measurement of X-ray diffraction, bone powder was held on a slide glass using an adhesive tape. To keep a wet surface of bone specimen, the physiological saline was often sprayed.

6. RESULTS AND DISCUSSIONS

6.1 Bone Powder Measurements

Figure 8 shows a result of bone power measurement, which is relationship between grain size of bone powder and the peak diffracted angle 2θ. Each close circle shows the mean values and the range of bar is the standard deviation of ten measurements. From the figure, it is clear that the peak position of X-ray diffracted diagram shifted to lower angle side with smaller particle size below 500 mm. Because 2θ of only HAp is measured in this method, this behavior means that the microscopic HAp structure is released from the restriction of collagen at HAp-collagen composites in bone tissue. In this experiment, the peak angle of particles with mean diameter below 45mm was defined as a non-strained state. This value was $2\theta_0$ = 14.521 deg.

6.2 Residual Stress of Bone Axial Direction

Figure 9 shows the variation of residual stress for bone axial direction from intact state CO to C5 specimen. These results are the mean values measured from three specimens. CO specimen could be measured only at the intermediate-medial portion of tibia where the thinnest of soft tissue as skin and muscle was observed. The residual stress for bone axial direction was the value of 10.5 MPa. By removing the soft tissues, this stress was released to almost zero. C1 specimen showed in the proximal region that the tensile stress existed in the medial region, while compressive stress existed in the other region. In the intermediate region the compressive stress existed in lateral region. In the distal region, the tensile stress existed at anterior, and the compressive stress at lateral and medial region. In comparison with C1 and C2, we can know the influence of cutting fibula as an indeterminate structure (Tanaka and Adachi, 1996). However, almost no variation of residual stress can be seen without proximal-posterior, intermediate-medial and distal anterior region. C3 specimen after removing both epiphyses showed that tensile stress increased in proximal-medial region, and compressive stress increased in distal-medial region. C4 specimen cutting three parts (proximal, intermediate and distal) showed that stress increased in tensile direction in

every portion. C5 specimen of four parts cut from C4 showed that tensile stress at proximal-medial and posterior was released, but increased at distal-posterior and medial. Therefore, by sequential cutting experiment, the influence of bone structure on residual stress in bone axial direction can be confirmed at every region of rabbit's tibia.

6.3 Residual Strain of Circumferential Direction

Residual stress for circumferential direction was not obtained in this work, since a very small specimen for four points bending test needed to measure material constants on this direction and it could not be made. Therefore, only residual strain in this direction was measured and discussed. Figure 10 shows the residual strain for circumferential direction. The compressive strain of 18 micrometer was obtained at C0 specimen in intermediate-medial region. C1 specimen excluded soft tissue showed tensile strain at proximal-posterior and medial region, and compressive strain at proximal-lateral, while no strain was at anterior region. In intermediate region, tensile strain existed at anterior. C2 specimen cutting of fibula showed that strain changed to tensile at proximal-medial and intermediate-anterior region, but increased to compressive at proximal-lateral and intermediate-anterior. In case of cutting both epiphyses, the residual strain at every region without proximal-posterior was released. C4 specimen showed that strain was released at proximal and intermediate region. C5 specimen showed that the strain was released at every region.

Therefore, from this sequential cutting test, the variation of residual stress or strain at lateral and anterior portion was relative small. The effect of fibula cutting is confirmed in posterior at axial direction and in posterior to medial portion at circumferential direction. The sequential cutting process gradually released circumferential residual strain at medial and posterior portion.

7. CONCLUSIONS

1. The X-ray diffraction method could be presented as effective experiments to measure residual stress/strain in bone structure.
2. From this method, residual stress/strain existing in bone structure could be measured in the process of sequential cutting.
3. By the measurement of bone powder, it was confirmed that residual strain mainly induced from HAp-collagen composites.
4. Residual stress for bone axial direction is relatively small and has little dependency for sequential cutting, while circumferential residual strain at medial and posterior portion was gradually released by sequential cutting.

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